1. **Please replace the paragraph starting on page 4 line 5 and ending on page 4 line 13 with the following amended paragraph:**

The present invention provides a system for the high throughput screening of chemical compounds. The system is particularly applicable to analysis of compounds that affect biological processes. In preferred embodiments, the invention detects events that occur inside cells. For example, the inventive system may be applied to the detection of compounds that alter the intracellular concentration of a target biological compound. Alternatively or additionally, the inventive system may be employed to identify compounds that suppress or enhance a specific biological phenotype. In preferred embodiments, the compounds analyzed comprise compounds synthesized by combinatorial chemistry.

2. **Please replace the paragraph starting on page 5 line 17 and ending on page 5 line 23 with the following amended paragraph:**

In one preferred embodiment, the present invention screens chemical compounds for their effects on chemical and/or biological systems by detecting the presence or amount of a component present or produced by the system, in which the component acts as a marker for the chemical or biological process of interest. Preferably, the component is detected by means of its interaction with a binding partner ligand. Preferably, the binding is specific. In certain preferred embodiments, the binding partner ligand is an antibody.

3. **Please replace the paragraph starting on page 6 line 17 and ending on page 7 line 6 with the following amended paragraph:**

In another preferred embodiment, the present invention provides a system for identifying compounds capable of affecting a biological or chemical process comprising a high density array of reaction vessels containing at least 100 reaction vessels and an

assay solution containing at least one reagent for detecting levels of component in a biological or a chemical process or resulting from a biological or a chemical process. Preferably, the array of reaction vessels contains at least 300 reaction vessels, and/or each vessel has a volume less than or equal to approximately 50 microliters, and/or the assay solution includes a component that is detected using chemiluminescence. More preferably, the array of reaction vessels contains at least 1000 reaction vessels, each vessel has a volume less than or equal to approximately 2 microliters, and/or the detected chemiluminescent compound is produced by a peroxidase. Most preferably, the array of reaction vessels contains at least 5000 reaction vessels, each vessel has a volume less than or equal to approximately 250 nanoliters, and/or the peroxidase is horseradish peroxidase.

4. **Please replace the paragraph starting on page 7 line 15 and ending on page 7 line 17 with the following amended paragraph:**

The present invention further provides compounds and compositions that are useful as microtubule stabilizers and/or as specific effectors of the cytoskeleton, as well as methods for using such compounds and compositions.

5. **Please replace the paragraph starting on page 28 line 17 and ending on page 28 line 23 with the following amended paragraph:**

Preferably, the cells used in reaction vessels described in the preceding paragraph are mammalian cells. However, any biological or chemical system may be utilized in the reaction vessels in accordance with the present invention. For a non-limiting example of another biological system, other cells such as bacteria, yeast, plant and insect cells may be used. The number of cells for these examples that are used in miniaturized reaction vessels will differ from mammalian cells depending on the size of the cells.

6. **Please replace the paragraph starting on page 37 line 20 and ending on page 38 line 12 with the following amended paragraph:**

In one particular preferred embodiment of the present invention, the detectable entity comprises a peroxidase that catalyzes a chemiluminescent reaction. For example, a variety of chemiluminescent substrates are available for horse radish peroxidase (HRP). Preferred for use in the practice of the present invention are diacylhydrazides, such as luminol. Diacylhydrazides are oxidized in the presence of hydrogen peroxide, and luminesce to emit photons. The luminescence resulting from the oxidation of luminol can be enhanced using a phenol derivative, preferably 4-iodophenol (ECL™; Nycomed Amersham Corporation, Buckinghamshire, England). The luminescence can then be detected by film, detected using photomultiplier technology or detected by a charge-coupled device attached to a camera and/or a computer. The use of luminol as an HRP substrate greatly enhances the sensitivity of detecting HRP relative to other substrates such as color dyes (*e.g.* o-phenylenediamine; OPD). This increased sensitivity of detection allows for small sample sizes. Figure 1 presents a schematic representation of but one particular preferred embodiment of the present invention, in which HRP is coupled to a secondary antibody, used to detect a primary antibody that interacts with a detection target.

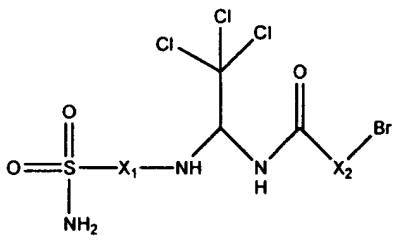
7. **Please replace the paragraph starting on page 69 line 16 and ending on page 69 line 22 with the following amended paragraph:**

We have used the inventive cytoblot system to identify compounds that alter the progression of mammalian cells through the cell division cycle. In particular, we have found one set of compounds that exibit the vinblastine-like property of destabilizing microtubules, one set of compounds that exhibit the taxol-like property of stabilizing microtubules, and one set of compounds that alter chromosome segregation in a novel fashion. Of particular interest are the microtubule destabilizing compounds.

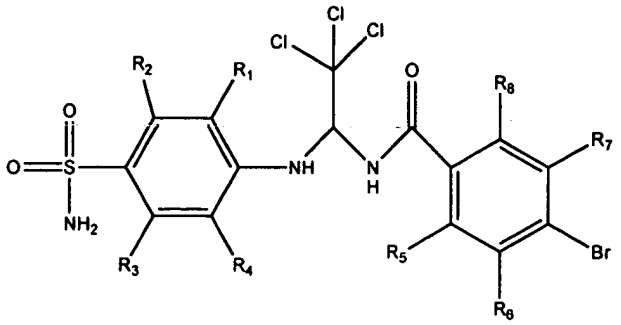
8. **Please replace the paragraph starting on page 69 line 23 and ending on page 70 line 9 with the following amended paragraph:**

In general, the present invention provides compounds and pharmaceutical compositions that alter the progression of cells through the cell cycle (see Figures 24-26). Compounds of particular interest are summarized in Figure 26. In certain preferred embodiments, the compounds and are capable of acting as inhibitors of the cell cycle. Specifically, these compounds are useful as microtubule stabilizers and as specific effectors of the cytoskeleton. In one aspect, the present invention provides novel compounds as shown by **(10)**, **(20)**, **(30)**, **(40)**, **(50)** and **(60)** below, and as described below. Furthermore, the present invention provides pharmaceutical compositions comprising a therapeutically effective amount of the compound having any one of the structures **(10)**, **(20)**, **(30)**, **(40)**, **(50)**, or **(60)**, associated with a pharmaceutically acceptable carrier.

9. **Please replace the chemical structure at the top of page 74 with the following amended chemical structure:**



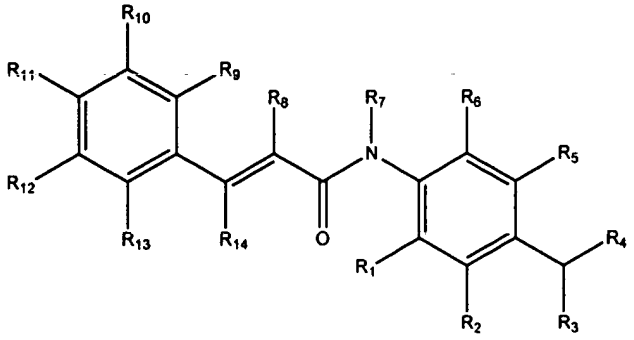
10. **Please replace the chemical structure at the top of page 75 with the following amended chemical structure:**



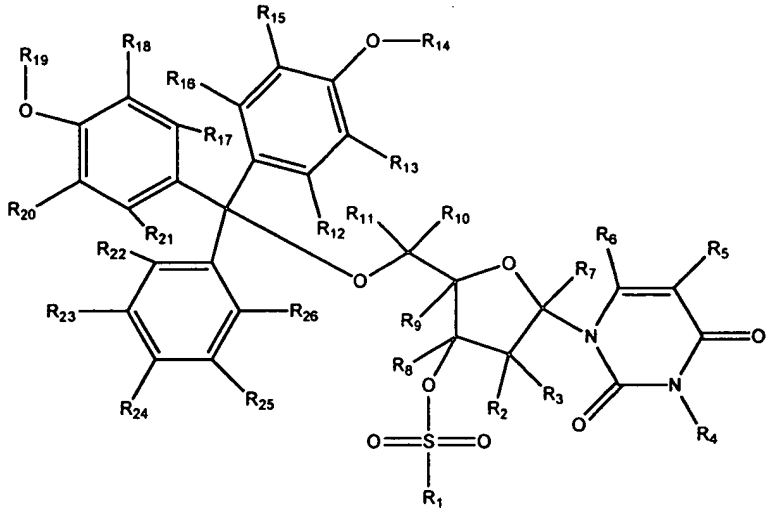
11. **Please replace the paragraph starting on page 75 line 4 and ending on page 75 line 16 with the following amended paragraph:**

R_1-R_8 are each independently the same or different and are selected from the group consisting of H, Br, Cl, F, NH_2, CO_2H, OH, linear or branched alkyl, linear or branched acylamino, linear or branched acyloxy, linear or branched alkoxycarbonyl, linear or branched alkoxy, aryloxy, linear or branched alkylaryl, linear or branched hydroxyalkyl, and linear or branched aminoalkyl or aryl group. The abovedescribed compounds represent novel compounds provided by the present invention, with the limitation that, in (20) above, R_1-R_8 cannot each simultaneously comprise hydrogen. Each of the abovedescribed compounds can be associated with a pharmaceutically acceptable carrier to provide novel pharmaceutical compositions, even when R_1-R_8 each simultaneously comprises hydrogen. In a particularly preferred embodiment, compositions are provided where R_1-R_8 are each hydrogen. Each class of compounds, as depicted by (10) and (20) above, affects the cell cycle by stabilizing microtubules.

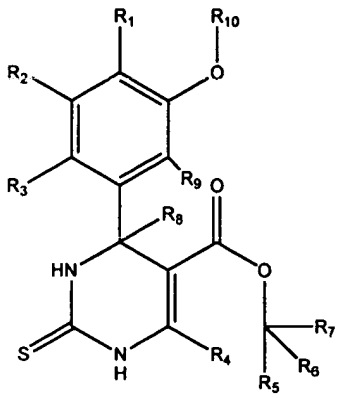
12. **Please replace the chemical structure on page 76 lines 5-9 with the following amended chemical structure:**



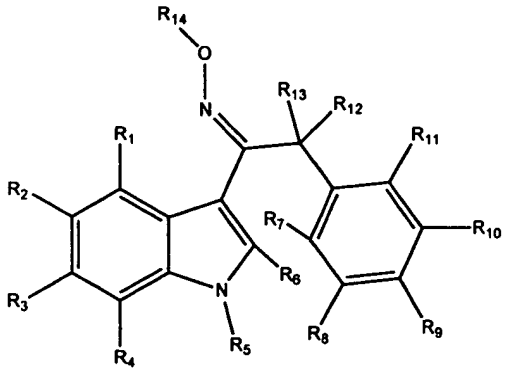
13. **Please replace the chemical structure on page 77 line 7 to the end of page with the following amended chemical structure:**



14. Please replace the chemical structure on page 79 lines 1-3 with the following amended chemical structure:



15. Please replace the chemical structure on page 80 lines 6-8 with the following amended chemical structure:



16. Please replace the paragraph starting on page 81 line 9 and ending on page 81 line 10 with the following amended paragraph:

Each of the compounds **(30)-(60)** shown above is capable of interfering with the cytoskeletal structure of cells undergoing mitosis.

17. **Please replace the paragraph starting on page 81 line 11 and ending on page 81 line 13 with the following amended paragraph:**

Furthermore, as will be appreciated by one of ordinary skill in the art, the present invention is intended to include all enantiomers and diastereomers of the inventive compounds utilized in the compositions and methods.

18. **Please replace the paragraph starting on page 81 line 16 and ending on page 82 line 2 with the following amended paragraph:**

The compounds disclosed herein inhibit cell cycle progression by either 1) acting on microtubules or 2) effecting the mitotic cytoskeleton, and thus may be used to treat a variety of human conditions including a broad range of cancers and pathogenic infections. As noted above microtubule stabilizing agents may be used to prevent or reduce atherosclerosis or restenosis. Furthermore, compounds of the present invention may be used as immunosuppressants or as morning-after pills. Thus, the present invention provides pharmaceutical compositions comprising any one of the abovedescribed compounds **(10)**, **(20)**, **(30)**, **(40)**, **(50)**, or **(60)** and a pharmaceutically acceptable carrier. Methods for treating disorders are also provided comprising administering a therapeutically effective amount of an inventive composition to a patient in need.

19. **Please replace the paragraph starting on page 84 line 5 and ending on page 84 line 18 with the following amended paragraph:**

Clearly, compounds that act as cell cycle inhibitors are invaluable to the study of the cell cycle pathway. In general, inhibitors of cell cycle progression are essential as tools that can be used to achieve arrest at specific points in the cell cycle. This allows one to administer the reagent to a population of cells to achieve synchronization of the mitotic cell cycle. In addition, specific proteins or activities may be identified as being essential to cell-cycle-related functions by their interaction with small molecule

inhibitors of the cell cycle. Proteins that play an important role downstream of the direct target may be confirmed by indirect inhibition by the same agent. In essence, exposure of cells to such reagents causes a conditional loss of function in the target protein in a similar manner to that achieved by the use of temperature-sensitive mutations in a gene. Similarly, such inhibitors of microtubule polymerization and depolymerization may be used to identify new cytoskeletal proteins and unravel the function and regulation of cytoskeletal proteins.

20. Please replace the paragraph starting on page 84 line 19 and ending on page 85 line 14 with the following amended paragraph:

Additionally, as will be appreciated by one of ordinary skill in the art, any in vitro assay may be used to monitor inhibition at different mitotic transition points, when the cell cycle progresses from one phase to the next. This may be accomplished by altering the timing of addition of the chemical compound in question to the mitotic extract. Alternatively it may be desirable to test whether certain compounds inhibit mitosis at early transition stages (*e.g.*, prophase or anaphase). According to one preferred embodiment of the present invention, the test compound is added to the interphase mitotic extract simultaneously with the Delta90 cyclin protein (and thus at the onset of mitosis) to test for successful of inhibition of early transition stages. Another aspect of the invention tests whether certain compounds inhibit mitosis at late transition stages (*e.g.*, microtubule assembly and disassembly and chromosome segregation). Thus according to other preferred embodiments of the present invention, the test compound is added after Delta90 cyclin so that mitosis has progressed past the early transition stages and inhibition of ubiquitin degradation can be assessed. Effectors of microtubule stability are particularly desirable compounds according to the present invention. Identification of such compounds is likely to allow further dissection of key regulatory steps of the mitotic pathway, cytoskeletal organization and serve as important tool in various other research and therapeutic purposes as mentioned above.

21. **Please replace the paragraph starting on page 91 line 13 and ending on page 91 line 19 with the following amended paragraph:**

In agreement with the phenotypic effects of synstab A observed through fluorescence microscopy, fluorescence-activated cell sorting confirmed that, similar to cells treated with nocodazole or taxol, cells treated with synstab A had fully replicated chromosomes (4N DNA content) and increased TG-3 staining. In addition, immunoblotting of total cell extracts derived from cells treated with taxol or with synstab A at concentrations that do not affect viability show increased TG-3 reactivity.

22. **Please replace the paragraph starting on page 92 line 7 and ending on page 92 line 24 with the following amended paragraph:**

We investigated the phenotype of mammalian cells (BS-C-I) treated with the compounds of group III . Live images were taken as described previously (Cramer *et al.*, *Curr. Op. Cell Biol.* 6:82, 1994); for immunofluorescence, the cells were stained with a Golgi-specific antibody (anti-Golgi 58K protein antibodies [Sigma]) or with anti-α-tubulin antibodies (DM1 A [Sigma]); actin was visualized using TRITC-conjugated phalliodin (Sigma); lysosomes were stained with LysoTracker (Molecular Probes; Palmiter *et al.*, *EMBO J.* 15:1784, 1996). Our examination of the distribution of microtubules, actin, and chromatin in fixed cells by fluorescence microscopy allowed us to divide the small molecules into three classes. Twenty-seven had no observable effect on the microtubule and actin cytoskeleton or on chromosome distribution. Consistent with the data from the cytoblot assay we observed an increase in the number of normal appearing mitotic cells. These compounds may increase the mitotic index by perturbing the function of proteins that regulate progression through the cell cycle, *e.g.*, anaphase regulators, rather than structural or mechanochemical components of the mitotic spindle. It is also possible that these compounds have a subtle effect on cytoskeletal dynamics or chromosome organization that may not be observable in fixed cells.

23. Please replace the paragraph starting on page 96 line 9 and ending on page 97 line 6 with the following amended paragraph:

We refer to this approach of phenotype-based small molecule screening as chemical genetics, because of its conceptual similarity to classic forward genetic screens. The cytoblot assay will be a key tool for chemical genetics. Using appropriate antibodies, it can provide a quantitative readout of essentially any post-translational modification of a specific protein in the cell. In this Example, a cytoblot assay for phosphorylation of nucleolin was used as a readout of mitosis, and our screen detected compounds that arrest cells in mitosis. After eliminating compounds that targeted pure tubulin, a sufficiently small number of the original 16,320 compounds remained for us to use a systematic visual analysis. For monastrol, the information from such analyses facilitated the identification of the kinesin Eg5 as a cellular target. Previously the only known small molecule kinesin inhibitors were 5'-adenylylimido-diphosphate (AMP-PNP) (Saxon, *Met. Cell Biol.* 44:279, 1994) and a marine natural product (Sakowicz *et al.*, *Science* 280:292, 1998), both of which are not cell-permeable and affect multiple kinesin family members. Monastrol, in contrast, is the first example of a cell-permeable compound that selectively perturbs the function of a motor protein essential for mitosis. Other motor proteins involved in lysosome and Golgi distribution seem not to be affected by other mechanisms have shown anti-tumor activity in humans (Jordan *et al.*, *Met. Enzymol.* 298:252, 1998), monastrol may serve as a lead for anti-cancer drugs. Monastrol will, however, be a valuable tool for dissecting the function of Eg5 in the establishment of spindle bipolarity and other cellular processes.

24. Please replace the paragraph starting on page 102 line 14 and ending on page 103 line 17 with the following amended paragraph:

LUCIFERASE ASSAYS: Our transient transfection luciferase assay was described previously by us (Stockwell *et al.*, Chem Biol 5:385, 1998). Briefly, 100,000 Mv1Lu mink lung epithelial cells were transiently transfected in 12-well dishes with 400 ng

p3TPLux or pNFkB-Lux, with or without 50 ng pFC-MEKK, in 300 µL minimal essential medium with non-essential amino acids. The DEAE-dextran/chloroquine/DMSO method was used for transfection (Stockwell *et al.*, Chem Biol 6:71, 1999). After cell lysis in 120 µL lysis buffer, a Beckman LS 6500 liquid scintillation counter was used in single photon mode to quantitate luminescence. For detection of luciferase activity in 6F cells (including the primary screen), 20,000 6F cells were seeded in 50 µL of 10% mink medium in each well of a white 384-well plate (Nalge Nune International; Naperville, IL; cat#164610) using a Multidrop 384 liquid dispenser (Lab Systems; Helsinki, Finland). After 16 hours, medium was removed using a 24 channel wand (V&P Scientific, Inc.; San Diego, CA; cat#VP186L), the cells were washed with 75 µL of 0.2% mink medium (containing 0.2% FBS), and reagents were added in 40 µL of 0.2% medium. For the primary screen, reagents were added by pin transfer using 384 polypropylene pin arrays (Matrix Technologies; Hudson, NH). After 24 hours, the cells were cooled on ice and washed twice with 75 µL Hanks Balanced Salt Solution (HBSS; GibcoBRL; cat#24020-117). Then 20 µL lysis buffer (25 mM glycylglycine (Sigma; cat#E-0396), 1% Triton X-100 (Sigma; cat#T-9284), 1mM dithiothreitol (DTT; Sigma; cat#D-5545), 1mM phenylmethylsulfonyl fluoride (PMSF;Sigma; cat#P-7626)) was added to each well with a Multidrop. After incubating the cells for five minutes on ice, 20 µL of ATP/luciferin solution was added (25 mM glycylglycine pH 7.8, 15mM $MgSO_4$, 4 mM EGTA, 6.25 mM K_2HPO_4 (Sigma; cat#P5504) pH 7.8, 5mM DTT, 75 µM D-luciferin (Sigma, cat# L-9504, 2mM ATP (Sigma; cat#A-7699)). Light output was immediately measured with an Analyst 384-well platereader (LJL), with 0.5 second counting time per well.

In the Claims

Please cancel claims 39-56 and replace them with new claims 57-81, as presented below.

57. A method for screening one or more test compounds to identify at least a subset of those